

Augmentation of Demineralized Bone Matrix Post-Tooth Extraction Increases The Density of Gingival Collagen Fiber of Rabbit Mandible

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Abstract

Most cases report that the abnormality of bone defect is engendered by tooth extraction. The powder of demineralized bone matrix (DBM) is required to fill alveolus bone for reconstructing material or preventing tissues defect after tooth extraction. The aim of this study was to determine the density of gingival collagen fiber on wound healing after the augmentation of DBM following the extraction of incisivus tooth. In this study, 36 male rabbits aged 2.5-3 months weighing 900-1,100 grams were randomly divided into two groups: control and treated rabbits. The incisivus teeth of mandibles of treated rabbits were extracted and augmented with the allograft DBM powder. The gingival was sutured with non-absorbable sutures. The same procedures were employed to the control group, except that these rabbits were augmented with DBM powder. Subsequently, the rabbits were sacrificed on days 1, 3, 5, 7, 10, and 14 after surgery, and each observation was represented by three rabbits in the sample. The gingival (ca. 0.5-1cm) was cut and fixed immediately in 10% paraformaldehyde. The staining was done using van Geison. In the treated rabbits, the density of gingival collagen fiber significantly increases in all observation days except on first day, indicating that the allograft powder of DBM successfully accelerates the wound healing process.

Keywords: Demineralized bone matrix, collagen fiber, transplantation, augmentation.

1. Introduction

Transplantation purports to reconstruct parts of body that are defect due to traumas or diseases. Trauma happening in mouth hollow is primarily rendered by the effect of tooth extraction. This may cause damage to alveolus bone; for instance, a finding reported by Mercier¹ shows that bone abnormality in the realm of dental surgery is mainly damage to bone after tooth extraction. Treatment or prevention of bone damage can be undertaken by giving bone graft for augmentation to induce the growth of new bone.^{2,3}

Marx *et al.*⁴ report that the graft contains conductive matrixes, inductive cells, and factors that physiologically play a role in the healing process. Success in graft augmentation requires several prerequisites^{2, 3, 5} eliminating immunogenic components that basically contain protein, being free from infection, being absent from bone marrow, and not having systemic diseases.

Demineralized bone matrix (DBM) is a dry and coagulated allograft that can be stored in room temperature. Its biochemical character is slightly decreasing since the drainage utilizes a coagulated approach and the sterilization harnesses Gamma (γ) ray. This graft has an advantage of alleviating the

prevalence of immune reaction and of being user friendly. Previous intensive research has provided evidence of DBM's ability to induce the formation of bone ^{6,7}. Tandelilin *et al.* ⁸ find that DBM induces the density of the growth of bone collagen fiber. DBM also enhances the density of osteoblast ⁹. DBM powder is composed of collagen and non-collagen contents. According to Wozney ¹⁰, DBM allograft contains bone morphogenic protein (BMP), platelet derived growth factor (PDGF), and transforming growth factor (TGF- β).

Brunsvold and Mellonig ¹¹ indicate that DBM is beneficial in the rehabilitation process following jaw resection, jaw fracture, jaw cyst extirpation, pre-prosthetic surgery, augmentation around tooth implant, and augmentation of bone damage due to abnormality in periodontal tissue, augmentation of alveolar process resorption on a toothless jaw, augmentation post-extraction of many teeth, and augmentation of alveolus post-odontectomy. For a simple case aimed at filling a cavity, only cancellous cubical, cancellous chip, or powder are needed.

Wound healing is basically a process or effort to overhaul damages to both hard tissues and soft tissues. In other words, this healing process is a response to the prevalence of laceration, physically and chemically, to tissues in the mechanism of structural maintenance such that the tissues' functions return to be normal ¹². Peterson *et al.* ¹³ reveal that physical laceration to soft tissues can take forms of little or massive surgical incision, pressure, extreme changes in temperature, irradiation, and blockage in artery and vena flows. Physical laceration to hard and soft tissues can occur simultaneously; for instance, in the case of wound post-tooth extraction.

Wound healing embraces a series of processes, such as chemotaxis, cell division, neovascularization, synthesis of new ECM components, formation and reformation of scraped tissues. Dissolved mediators such as growth factor, cytokine, matrix metalloproteinase (MMP) and its regulator are able to control stages involved in wound healing through their effects on several cells and extra-cell membrane henceforth called ECM ¹⁴.

Acute wounds triggered by tissue laceration involve several phases. The physiology of natural acute wound healing occurs in three phases: inflammation, proliferation, and remodeling or maturation. Those three phases will proceed normally so long as no disturbances prevail, both internally and externally. The main component in the healing process of soft tissues is collagen alongside epithelial cells. Fibroblast is a cell responsible for collagen synthesis. Final result of synthesis and degradation yields the remodeling of skeleton of bundle tissues—an important step in chronic inflammation and wound healing ¹⁵.

As widely known, collagen is a protein most found in the body and composed of glycine amino acid of 33.5 %, proline of 12 %, proline hydroxyl of 10%, and other amino acids. In human body, collagen is the most found protein, constituting 30% to the total protein ¹⁶. Prockop and Kivirikko ¹⁷ find evidence that the types of collagen have reached more than 20 categories spread throughout human body. However, the most found and learned are types I, II, III, IV, and V. Type I collagen is the most found and widely spread. It prevails as the structure in tissues, hence is labeled as collagen fiber. In addition, it is also extant in the formation of bone, dentin, tendon, dermis, and gingival ^{16,18}. Alongside type I collagen is also found type III collagen in gingival ¹⁸.

Collagen fiber consists of extremely delicate fibers. According to Fawcett ¹⁹ this fiber looks white when bundled with sheaves. Collagen fiber comprises collagen protein with its molecules composed of three α polypeptide chains and stacked in the form of triple helix ²⁰. Collagen is produced in endoplasmic reticulum of fibroblast cell in the form of procollagen, intermediated by vesicle and secreted from cell surface. Subsequently, procollagen will be polymerized to be tropocollagen that has a length of 280nm¹⁶. These molecules are then cross-linkingly stacked so as to form collagen fiber ²¹. Collagen fiber is strong and resistant to tension but flexible enough and collagen bundle often looks wavy ²². Net accumulation of collagen hinges on both synthesis and collagen degradation. Excessive formation of collagen will also be harmful as it may press and damage its original tissues or can form colloid ²³. This

study purports to observe the effect of demineralized bone matrix fiber augmentation in wound healing post-tooth extraction on the density of gingival collagen in rabbits' mandibles.

2. Materials and Methods

This study utilizes 36 male rabbits weighed 900-1,100 grams and aged 2.5-3 months. This sample was randomly divided into two groups: control and treatment groups. Control group then underwent extractions of bottom incisivus teeth, which was then augmented by DBM powder (370-710 μ M). The making and augmentation of DBM, analgesic treatment, and caring mechanisms followed the approaches of Tandelilin *et al.*⁸ and Tandelilin⁹. Subsequently, non-absorbable thread No. 0-5 was used to close the gingival. The same treatment was given to the control group without DBM augmentation.

Next, the rabbits were killed, with previously being put to sleep, on days 1, 3, 5, 7, 10, and 14 post-surgery, and each group was represented by three rabbits. Gingivals with an approximate width of 0.5-1cm² in the extraction area for all specimens were taken and fixated in paraformaldehyde solution 10%. This process continued with the making of Van Geison glassslide inventory to see the density of collagen fiber.

Light microscope with 400x magnification was used to observe the density of collagen fiber in gingival. This observation was based on grading determination: grade 1 for thin collagen density, grade 2 for medium density, and grade 3 for thick density (Figure 1). Subsequently, this was examined and analyzed using Man-Whitney test between treatment groups based on observation days.

3. Results

Means and standard deviations of the density of gingival collagen fiber for treatment and control groups based on observation days are summarized in Table 1. Next, Figure 2 shows the increasing pattern of the density of collagen fiber growth based on results in Table 1. The formation of collagen fiber has

been seen histologically since day 3, but this is less obvious for control group in which fiber spiral has yet to appear and fibers are still distant. This condition is different from DBM group that has attained the formation of collagen fiber bundles.

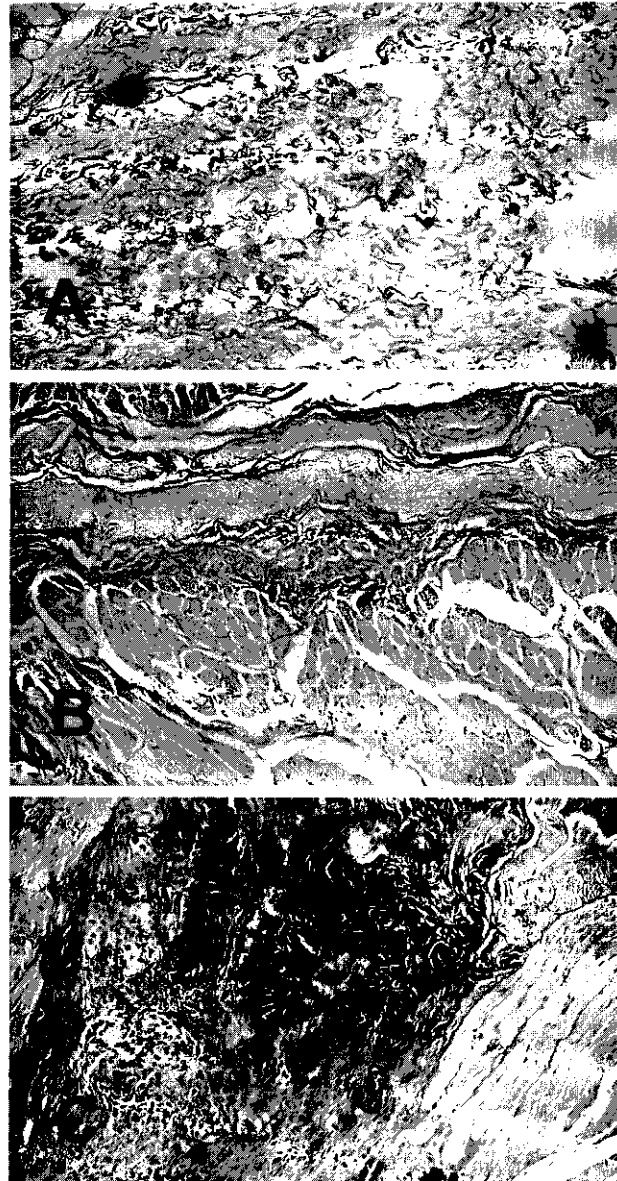


Figure 1. a) Thin collagen density (grade 1); b) Medium collagen density (grade 2); c) Thick collagen density (grade 3).

The original color version of the figure can be downloaded from the online version.

The collagen fiber bundles increase in number and density on day 5 after the prevalence of wound. The control group shows a density classified as less than medium category (Figure 3), observed at 400x zoom. This circumstance is indicated by the closer distance among fibers. The existence of collagen fiber bundles begins to appear on days 7 and 10. This differs from the DBM group that has been dense on day 7 and keeps increasing so as to look extremely dense on day 14.

Table 1. Means and standard deviations of the density of gingival collagen fiber for control group and DBM based on observation days (n =30)

Day	Mean \pm SD	
	Control	DBM
1	1,20 \pm 0,40	1,13 \pm 0,34
3	1,56 \pm 0,56	2,06 \pm 0,58
5	1,83 \pm 0,69	2,56 \pm 0,50
7	2,16 \pm 0,69	2,66 \pm 0,47
10	2,20 \pm 0,61	2,86 \pm 0,34
14	2,43 \pm 0,50	3,00 \pm 0,00

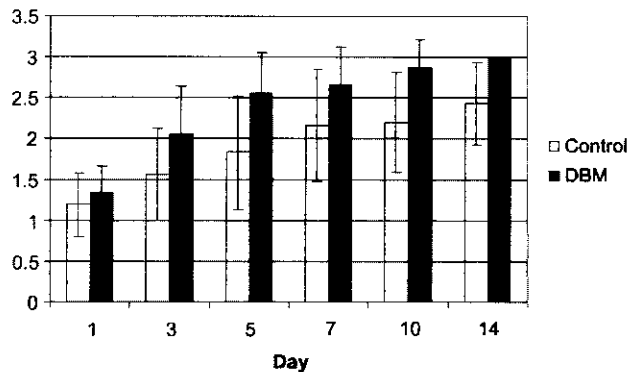


Figure 2. The increasing pattern of the density of gingival collagen fiber based on observation days for control group and DBM.

In general, clinical description on day 1 post-extraction shows inflammation alongside white layer of thin fibrin surrounding the area of wound in gingival. Three days after surgery, the DBM group shows the appearance of granulation tissues that

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look like pink tissues, and they can be distinguished from surrounding tissues. The wound is still concave and has yet to be closed perfectly by granulation tissues, except for the sewn area. Meanwhile, the wound edge is still partly white. This condition has not looked apparently different from that of control group whose necrotic tissues are still dominant.



Figure 3. The density of collagen fiber as shown by the arrows for control group (A) and DBM group (B) on day 5 after the prevalence of extraction wound. In Figure B, it appears that collagen bundles have formed numerous (400x zoom).

Five days post-surgery, the DBM group shows the prevalence of granulation tissues that appear to be pink tissues and are easily distinguished from surrounding tissues. Wound trace is still slightly concave and has yet to be perfectly closed by the granulation tissues. Some specimens are found with no fibrin tissues. For the control group, granulation

The original color version of the figure can be downloaded from the online version.

tissues have formed although wound still appears to be very concave. On day 7 post-surgery, rabbits augmented by DBM show that granulation tissues have closed perfectly. Fibrin is not seen any longer, and layer color has been difficult to differentiate from surrounding tissues. For the control group, wound still appears to be concave and has yet to close perfectly.

On day 10 post-extraction, the control group shows that wound has closed, and part of the tissue color has been difficult to differentiate from surrounding tissues. For the group augmented by DBM, it is observed that gingival has united with respect to color with surrounding tissues and gingivals. This is getting very obvious on day 14 when the process is completed such that no distinction can be seen in the edge of the wound. For the control group, although the wound has closed, it has not reached a perfect unity with surrounding gingivals. Subsequently, statistical analysis of the density of gingival collagen fiber is shown in Table 2 below.

Table 2. Significance of the density of gingival collagen fiber for control group and DBM group based on observation days (n=30).

Day	Significance
1	0,492
3	0,002**
5	0,000**
7	0,004**
10	0,000**
14	0,000**

Result shows in Table 2 indicates that there is a significant difference in the density of gingival collagen fiber between the two groups on every observation day, except on one day post-extraction as this involves previous collagen that has formed. The finding also implies that DBM is capable of inducing collagen biosynthesis in gingival. Hence, this differs from the case of bone collagen fiber, which is only significantly different on days 10 to 14 after tooth extraction.

4. Discussion

A series of events such as chemotaxis, cell division, neurovascularization, the synthesis of new ECM components, as well as the formation and remodeling of scraped tissues are phases involved in the wound healing process. In the process of re-epithelization, stroma cell migration, neoangiogenesis, and tissue remodeling, ECM proteolytic degradation is needed. The existence and development of inflammation in the DBM-augmented group in this research appears to be quicker, indicating a good response to the next phases in the wound healing process. It is apparent that growth factors contained in the DBM have functioned perfectly through synergic cooperations with recipient cells. Cell proliferation mechanism involved in the wound healing has occurred through transduction signals. The signals conveyed by growth factors are received by target cell cores such that the primary messengers are then tied with the growth factors. Albert *et al.*¹⁵ and Cotran *et al.*²⁴ report that the tie, which occurs through phosphorylation process, will activate receptors that in turn will trigger second messenger mechanism in cytoplasm with kinase protein. The prevalence of this mechanism causes extracellular signals to change into intracellular signals that can enter into cell cores. These signals are attached to certain parts in DNA that subsequently lead to transcription process, and further translation process then yields protein. Accordingly, cells will embark on the cyclical stage of cells to proliferate.

Proliferation phase in the healing process is very important, and signs corresponding with this phase include the prevalence of: re-epithelization, angiogenesis, and granulation tissues. Clinical observation results show that the formation of granulation tissues has begun three days after the extraction wound in the DBM group. The granulation tissues are composed of scaffolds filled with fibronectin, fibroblast, blood capillaries, and increasing amount of collagen type I and type III. During the formation of granulation tissues, fibroblast is stimulated by TGF- β for creating new ECM protein. Myofibroblast apoptosis and vascular cells intermediate the transition of granulation

tissues to become scraped tissues. The final result of this is a remodeling, which is synthesized collagen²⁵.

Retepeg begins appearing on day 5 in the healing process and continuously forms although it is still short and wide. The formation of retepeg indicates the beginning of maturity of epithelial cells. This circumstance has yet to be found in the control group. As the times passes, the DBM group shows barely distinguished tissue color from surrounding tissues after seven days. Meanwhile, the control group on day 7 shows wound that has closed but is still slightly concave, and it is found that one specimen has begun forming retepeg. After 10 days post-extraction, the DBM group in the healing process appears to have a more compact and longer retepeg reminiscent to a finger. This circumstance is attained by the control group on day 14 for only one specimen. Clinical figures show that the wound closure by granulation tissues is supported by microscopic pictures of retepeg, which is formed quicker in the DBM group. This indicates the ability of DBM to induce collagen biosynthesis and re-epithelization.

In general, wound healing process in mouth mucosa nearly resembles wound repair in coetaneous²⁶. The difference lies in the gingival wound where keratinocyte migrates directly to fibrin-fibronectin matrix without contacting with collagen^{26,27}. The wound healing in mouth mucosa is relatively faster, and scraped tissues are seldom forming in this case. In reality, the wound healing of mouth mucosa is reminiscent to the wound healing of fetal. Fibroblast in adult gingival is located in string tissue papilla, and some of its components share with the fibroblast of fetal. There are several factors in saliva, such as growth factor and ion (magnesium and calcium), that function to decrease some material components that may influence the gingival healing²⁷. The moisturization of mouth by saliva also functions to help heal wound.

Normal gingival commonly comprises collagen types I and III, but 99% of them embrace collagen type IV that is intertwined with lamina basal whereas 01% of them are produced by epithel. In this research, it is shown that the surge of the density of

collagen fiber occurs on days 5 and 7 in the DBM group. This finding is supported by Fonesca and Walker²⁸, who report that the formation of collagen fiber reaches its peak between those days. Statistical analysis on the DBM group significantly shows collagen formed is much denser for all sample based on observation days, indicating a good organization in ECM. As known, ECM physically provides an environment where cells live, and it functions as the scaffold of tissues and facilitates cell migration during wound recovery. The balance between synthesis and ECM's tight division is required by all tissues to function normally. The amount of composition of ECM is controlled by growth factors and mechanical pressure activities in tissues^{29,30}. Accordingly, it shows that DBM powder allograft as an augmentation material has an ability to hasten gingival reconstruction by inducing biosynthesis stimulation of collagen fiber and re-epithelization.

One of the parameters of gingival healing is the growth of collagen fiber. The effect of gingival's soft tissue healing augmented with DBM powder allograft is likely caused by the prevalence of various contained growth factors, such as PDGF and TGF- β , which synergically cooperate with recipient cells so as to influence the proliferation of cells involved in the gingival healing. PGDF mechanism as reported by Graves and Chonchran³¹ has shown the characteristic of pleiotropic effects on all wound repairs in the processes of tissue repairs and regeneration. PDGF is one of the factors that stimulates soft tissue healing³². It also functions as potential mitogen for string tissues' cells and to stimulate fibroblast chemotaxis, neutrophil, and macrophage³³. PDGF has an ability to activate microphage and to produce and secrete other growth factors that are crucial to various aspects in the healing process. PDGF stimulates fibronectin production³⁴ and hyaluronic acid³⁵. Expressions of various GDFs after the laceration in soft tissues enable the occurrence of regulation and regeneration processes in tissue repairs.

Previous research has provided much evidence that acidic and basic fibroblast growth factors stimulate DNA synthesis and cell replication³⁶ and

that bFGF hastens epithelization process³⁷. TGF- β has proved to be a strong promoting factor in the formation of ECM in several cell types. In the repairing process, TGF- β is cytokine that eliminates the recruitment of inflammatory cells³⁸. However, TGF- β can also function as chemotactic and mitogenic for neutrophil, lymphocyte, monocyte, macrophage, and fibroblast³⁹. The role that TGF- β plays during the proliferation process is its ability to stimulate angiogenesis and collagen deposition in tissues^{39, 40}. In the maturation phase of healing process, TGF- β plays a role in controlling excessive ECM components by conducting obstructing mechanisms on all substances that possibly divide or degrade them. In addition, it also plays a role in apoptosis process that prevails during wound maturation⁴¹.

5. Conclusion

Findings of this research conclude that DBM augmentation in the alveolus bone of rabbit's mandible after the extraction of incisivus tooth has significantly increased the density of gingival collagen fiber from three days on after the augmentation. Hence, DBM allograft is capable of hastening the wound healing process.

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